Single Molecule Detector

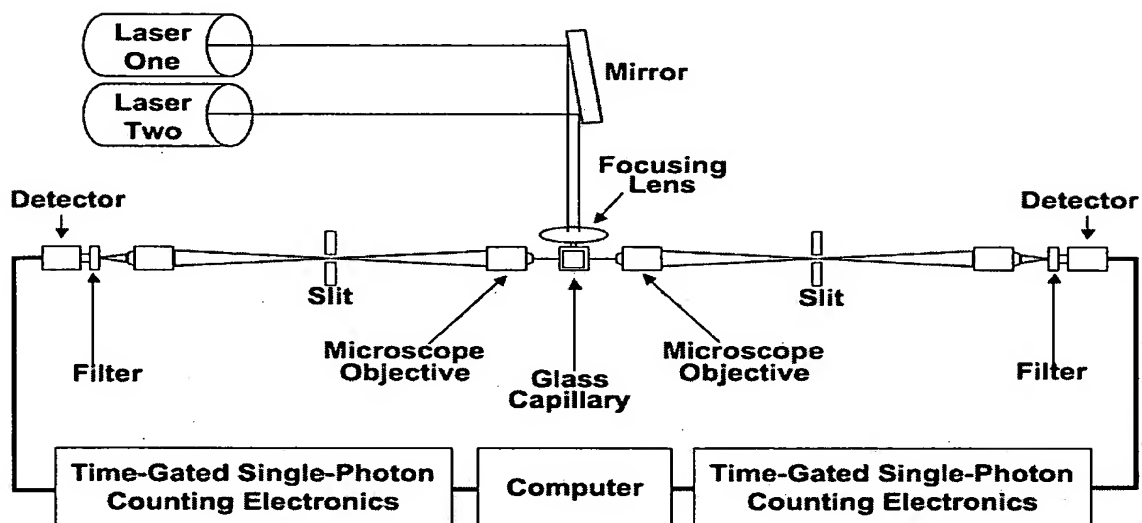Figure 1 Schematic diagram of the basic apparatus for single molecule detection using laser induced fluorescence.

Heart of the SMD Instrument

Glass Capillary Tube

Laser Beam #1
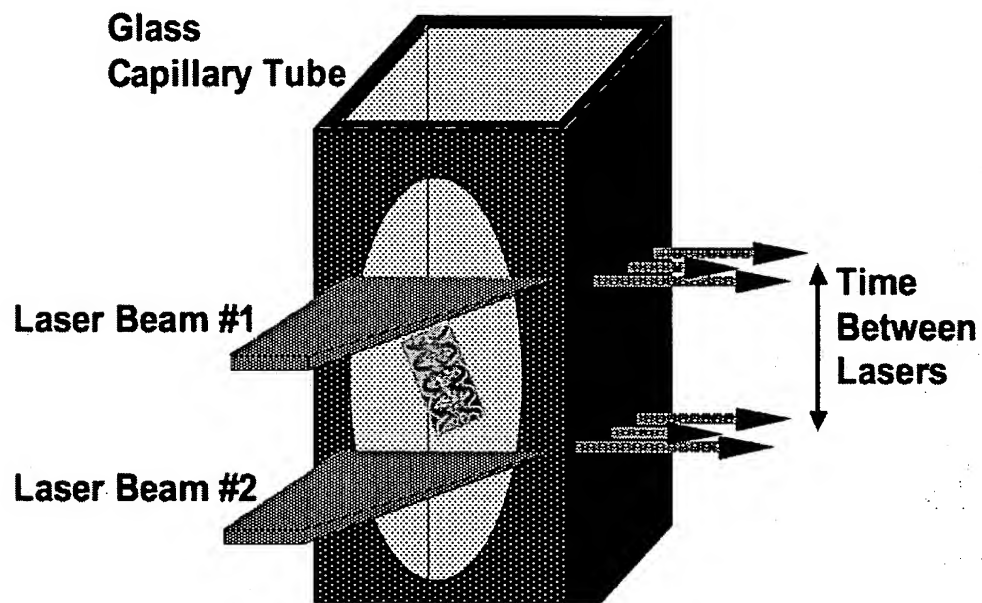
Laser Beam #2

Time Between Lasers

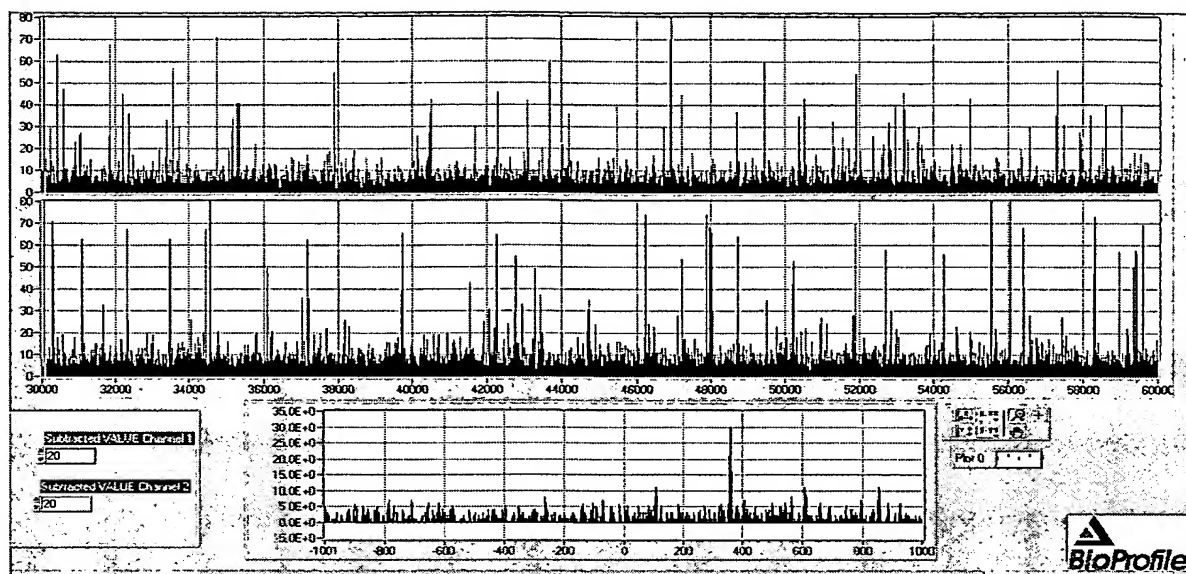Figure 2. Glass capillary tube forms the heart of the system.

Figure 3. Example of output from existing laboratory device. The upper two traces show the number of photons as a function of time (each unit represents 2 ms) for the two channels. Each large spike representsa fluorescence detection event over the background levels. The bottom trace represents the cross-correlation of the events for channel one with the events for channel two over a 30 sec period with a single peak at 700 msec

Figure 1.
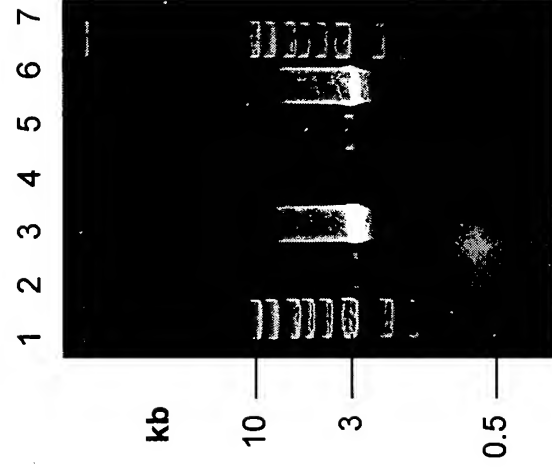
Ln1, 1kb DNA Ladder (500ng), ln2, 5 µL Seq. Id. No.1, 3 & 5 including 0.07mM Biotin-16-dUTP after dye coupling reaction; ln3, 5 µL Seq. Id. No. 2, 3 & 5 after dye coupling reaction; ln4, Empty ln5, 5 L Seq. Id. No.1, 3 & 5 including 0.07mM Biotin-16-dUTP after MinElute purification; ln6, 5 µL Seq. Id. No. 2, 3 & 5 after MinElute purification; ln9, 1kb DNA Ladder (500ng)

Figure 2. Sequence specific Single-stranded Probe Synthesis Reaction. ~10 ng of the 5230 base single-stranded probe was run on a on a 0.7% agaose TAE gel, stained with SybrGold Nucleic Acid Stain (Molecular Probes, Eugene, OR).
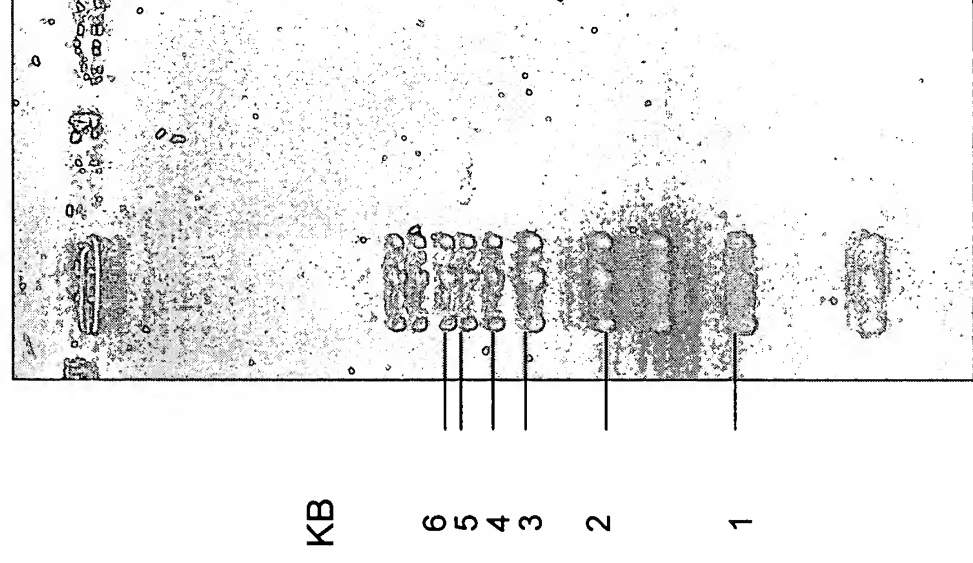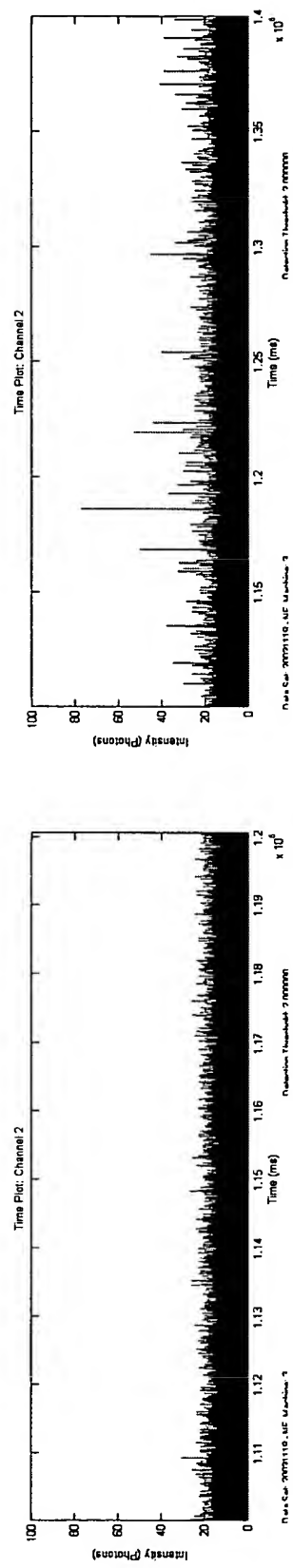
Figure 3. Dye Labeled Sequence specific Single-stranded Probe Detected on Single Molecule Instrument.

Single-stranded Probe labeled with dye

Buffer Only Control

Figure 4. Ligation of Oligo Adaptor Complex to Double Strand Tail

	Marker	1	2	3	4
447 bp HindIII Fragment		+	+	–	+
Oligo Adaptor Complex		+	+	+	–
Ligase		+	–	+	+

Figure 5. Ligated 450bp Probe Labeled with dye Detected on Single Molecule Instrument
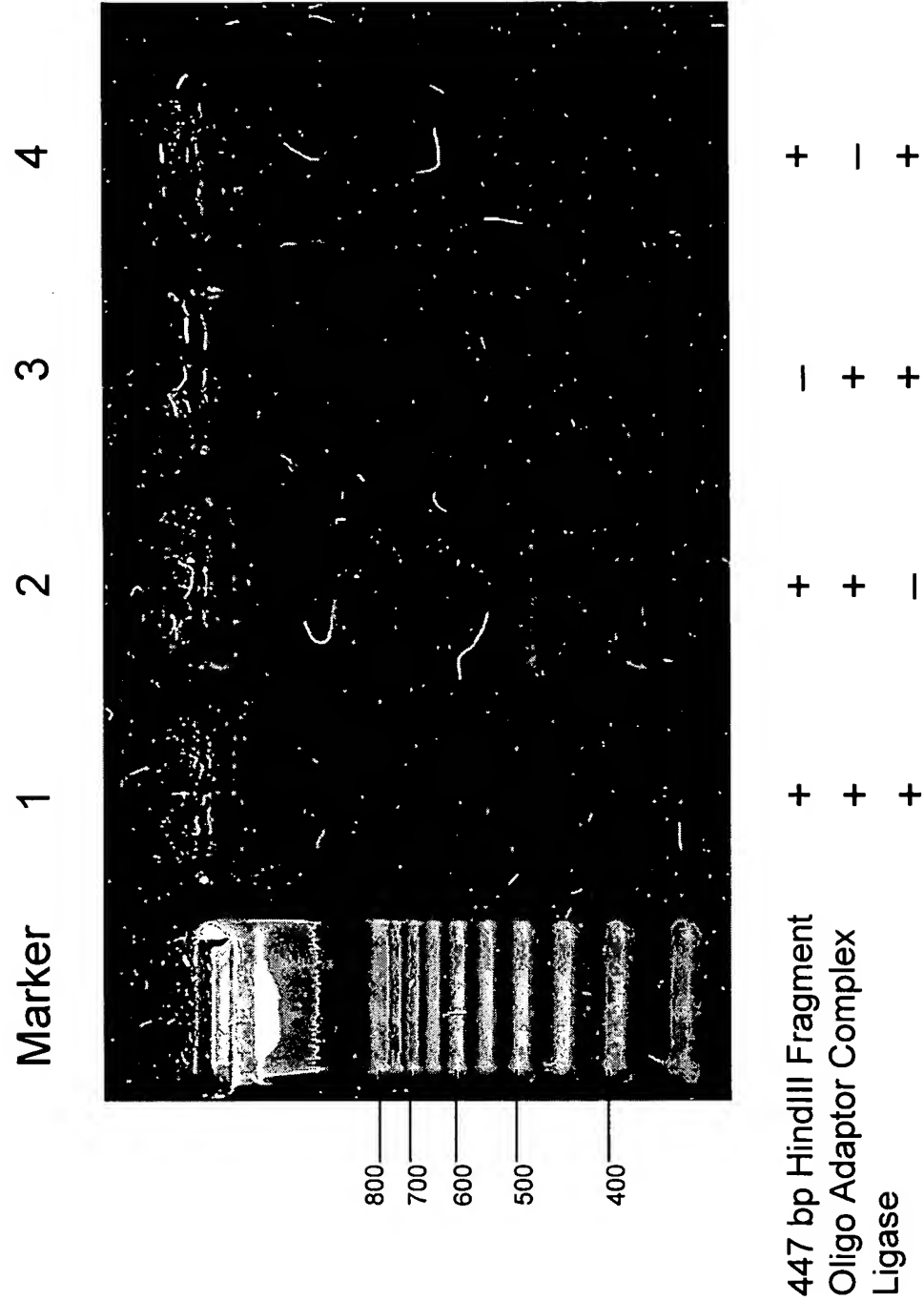

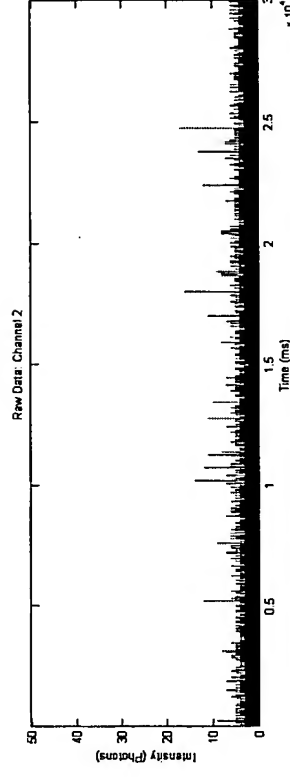Ligated 450 bp probe

Buffer Only Control

Figure 6. Variable Photon Intensities of Dye Labeled DNA Tails. Photon intensities are plotted over time for three samples. A. Buffer only control, shows system background to be ~ 5 photons. B. 1kb DNA labeled with dye shows bursts of 1-3 times that of the system background. C. 2.6 kb DNA labeled with dye shows bursts of 1-8 times that of the system background.

A. Buffer Only
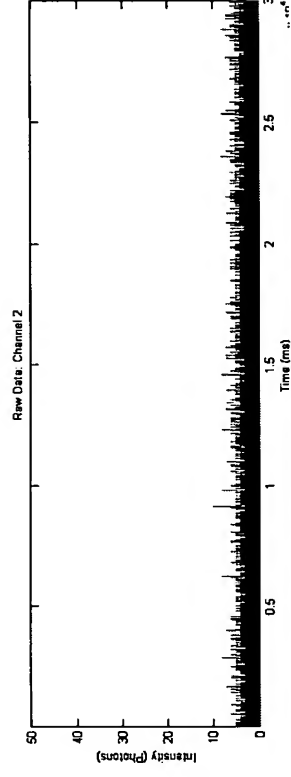
B. 1kb PCR Product Labeled with Dye

C. 2.6kb PCR Product Labeled with Dye